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98-414118/35 C06 D16 **PLBZ 97.01.20**
PLANT GENETIC SYSTEMS NV *WO 9831822-A1
97.01.20 97EP-200103 *(98.07.23)* C12N 15/82, A01H 5/00

Nematode-induced promoters from Arabidopsis thaliana line ARM1 - used to, e.g. prevent nematode attacks on plants, and to combat other plant pathogen(s) (Eng)

C98-125069 N(AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW) R(AT BE CH DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW)

Addnl. Data: KARIMI M, BARTHELS N, GHEYSEN G
98.01.19 98WO-EP00388

C(4-A8C2E, 4-E2E, 4-E4, 4-F8E, 14-B3A) D(5-H12A, 5-H12D5, 5-H14B3, 5-H16B) .5

An isolated DNA fragment comprises:
(a) nucleotides 1055 to 1417 of the 4160 bp DNA sequence given in the specification;
(b) nucleotides 46 to 408 or 46 to 573 of the 630 bp DNA sequence given in the specification;
(c) the 528 bp SspI-PvuII fragment of plasmid pARM1a (LMBP3638);
(d) the 3 kb PstI-StyI fragment of plasmid pch/ARM1D3500 (LMBP3635);
(e) the 3 kb PstI-StyI fragment of plasmid pch/ARM1D3500 and nucleotides 367 to 1190 of the 1273 bp DNA sequence given in the specification;
(f) the 3 kb PstI-StyI fragment of plasmid pch/ARM1D3500 and nucleotides 46 to 408 of the 630 bp DNA sequence given in the specification;
(g) the 2.5 kb PstI-SspI fragment of plasmid pARM1a3500 and nucleotides 46 to 408 of the 630 bp DNA sequence;
(h) the 1.3 kb SmaI fragment of plasmid pARM1a1300 (LMBP3636);
(i) the 1273 bp DNA sequence given in the specification;
(j) the 3.7 kb SmaI fragment of plasmid pARM1a3500, and
(k) a sequence which is 90% similar to the sequence of nucleotides 46 to 408 of the 630 bp DNA sequence.

Also claimed are:
(1) a chimeric gene comprising a plant-expressible promoter region comprising the above DNA fragment, a foreign DNA region, and a

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2' end formation and polyadenylation signal functional in plant cells;
(2) a plant cell comprising the chimeric gene of (1), and
(3) a plant comprising the chimeric gene of (1) integrated into its genome.

MORE SPECIFICALLY

The 630 bp, 1273 bp and 4160 bp DNA sequences are all promoter fragments from the *Arabidopsis thaliana* line ARM1.

USE

The chimeric gene of (1) can be used in a method for preventing nematode-attack of a plant. The DNA fragment can be used in a method for combating plant pathogens. The DNA fragment can also be used to express a gene in fixed feeding sites or specialised root cells of a nematode infected plant (all claimed).

The DNA fragment can be used against plant parasites nematodes including *Meloidogyne hapla, M. exigua, M. indica, M. javanica, M. africana, M. graminis, M. graminicola, M. arenaria, M. chitwoodii, Heterodera mexicana, H. punctata, H. cajani, H. glycines, H. oryzae, H. trifolii, H. avenae, H. carotae, H. cruciferae, H. goetingiana, Globodera rostochiensis, G. pallida, G. tabacum*, and those from the genera *Xiphinema, Nacobus*, and *Longidorus*.

ADVANTAGE

The promoters have enhanced specificity, and a shorter time of induction after infection, than currently available nematode-induced promoters.

PREFERRED MATERIALS

In the chimeric gene of (1) the foreign DNA region encodes a β-glucuronidase, a proteinase inhibitor, or a barnase. The plant cell of (2) further comprises a second chimeric gene comprising a barstar coding region under the control of a plant expressible promoter.

The plant of (3) is a potato plant, or an oilseed rape plant.

EXAMPLE

DNA was extracted from the *Arabidopsis thaliana* ARM1 line, using 0.2 to 2 g of plant material. The DNA pellets were dissolved in 400 µl TRIS EDTA to which 20 µg RNase was added.

After an incubation period of 20 mins at 37 °C, 400 µl CTAB buffer was added and the mixtures were further incubated for 15 minutes at 65 °C. The samples were extracted with 800 µl

chloroform/isoamylalcohol (24:1) and precipitated.

To determine the number of T-DNA inserted into ARM1, purified total plant DNA was digested with HindIII and EcoRI either alone or combined. Separation of the digested samples on 1% agarose gel in TAE buffer was followed by an overnight blotting to a Hybond-N membrane. The DNA on the membrane was fixed by UV cross linking. The 1.7 kb NruI fragment of pGUS1 comprising the coding region of the uidA gene was used as a probe. Radioactive labelling was performed.

The nylon membrane was incubated in a hybridisation buffer for 3 hours at 65 °C. Hybridisation was performed overnight in fresh hybridisation buffer to which the α^{32}P-dCTP labelled probe was added.

Further southern analysis, using several restriction enzymes allowed the determination that 2 intact T-DNA copies were present in reverse direction with the two right borders linked together and that a third incomplete T-DNA copy, integrated at a different locus were present. The T-DNA copy at the second locus does not comprise an nptII gene and segregates independently from the first locus which is responsible for the characteristic gus expression pattern. (DB)

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